


PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference AHBCP6230437		FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/GB2004/003273		International filing date (day/month/year) 28.07.2004	Priority date (day/month/year) 08.08.2003	
International Patent Classification (IPC) or national classification and IPC C12N5/00, C12N5/02				
Applicant CAMBRIDGE ANTIBODY TECHNOLOGY LIMITED et al.				
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input type="checkbox"/> sent to the applicant and to the International Bureau a total of sheets, as follows:</p> <p><input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>				
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>				
Date of submission of the demand 30.05.2005		Date of completion of this report 12.10.2005		
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized Officer Rojo Romeo, E Telephone No. +49 89 2399-7321		



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**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003273

IAP20 Rec'd PCT/PTO 07 FEB 2006

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This report is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:
 - ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

27 as originally filed

Claims, Numbers

1-50 as originally filed

Drawings, Sheets

1/14-14/14 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
 - ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
 - ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003273

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-50
	No: Claims	
Inventive step (IS)	Yes: Claims	1-50
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-50
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/GB2004/003273

Re Item V

**Reasoned statement with regard to novelty, inventive step, or industrial applicability;
citations and explanations supporting such statement**

1. Novelty (Art. 33(2) PCT)

Reference is made to the following documents:

- D1: WO 94/02592 A (CELLTECH LTD ; FIELD RAYMOND PAUL (GB)) 3 February 1994 (1994-02-03)
- D2: KOVAR J AND FRANEK F: "Iron compounds at high concentrations enable hybridoma growth in a protein-free medium" BIOTECHNOLOGY LETTERS, vol. 9, no. 4, 1987, pages 259-264, XP009037179
- D3: US-A-5 316 938 (RAPSON NICHOLAS T ET AL) 31 May 1994 (1994-05-31)
- D4: WO 92/05246 A (SMITHKLINE BEECHAM CORP) 2 April 1992 (1992-04-02)
- D5: WO 93/00423 A (NOVONORDISK AS) 7 January 1993 (1993-01-07)
- D6: NEUMANNOVA V ET AL: "GROWTH OF HUMAN TUMOR CELL LINES IN TRANSFERRIN-FREE, LOW-IRON MEDIUM" IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY. ANIMAL, TISSUE CULTURE ASSOCIATION, COLUMBIA, MD, US, vol. 31, no. 8, September 1995 (1995-09), pages 625-632, XP001118629 ISSN: 1071-2690
- D7: KEEN M J: "The culture of rat myeloma and rat hybridoma cells in a protein-free medium" CYTOTECHNOLOGY, vol. 17, no. 3, 1995, pages 193-202, XP009037173 ISSN: 0920-9069
- D8: DEMPSEY JONATHAN ET AL: "Improved fermentation processes for NS0 cell lines expressing human antibodies and glutamine synthetase." BIOTECHNOLOGY PROGRESS, vol. 19, no. 1, January 2003 (2003-01), pages 175-178, XP002298041 ISSN: 8756-7938

D1 discloses that, in agitated cultures, ferric ammonium citrate is toxic for hybridoma cells (ex. 2) and that at the concentration of 0.2 mg/l, ferric ammonium citrate kills myeloma cells (ex. 5). The medium used corresponds to the medium used in the present application. GS-NSO cells are also used, as in the present application.

D2 discloses the growth of hybridomas and parental myelomas in PFH medium without transferrin but with Fe-citrate (see Table. 2). The medium was also found to be suitable for

stirred suspension cultures (page 264).

D3 discloses media with an inorganic source of iron (0.25-5 mg/l; ferric and ferrous salts, e.g. ferric citrate or ferrous sulphate or ferric ammonium citrate). EDTA is used and it is mentioned that any iron source may be used which is not isolated from an animal source, for example chemical chelators. The medium without chelator would correspond to that of the present application (0.25-5 mg/l ferric or ferrous salt; claim 1) . It is used for CHO cells. Agitated (stirred) culture is contemplated (see column 6).

In claim 1 (e) of D4, there is the choice between 0 to 100 mg/l of a compound selected from the group consisting of transferrin, ferric fructose, ferrous citrate and ferrous sulfate. At page 3, it is said that the media described are not suitable for the culture of myeloma cell lines.

D5 discloses in example 5, that SP2/0 cells (a real myeloma cell line) can be cultivated in suspension in T-flasks (no agitation) with FeCl₃ and citrate. The highest iron concentration was 1000uM FeCl₃ corresponding to 0.16 g/L, the lowest of 30 uM corresponding to 48.72 mg/L iron.

D6 discloses the use of low (5 uM) and high (500 uM) in 96 well plates. The low ferric citrate concentration did not support the culture of the myeloma cells studied (nor hybridoma).

D7 discloses that the myeloma cell line Y0 grows in a protein free medium with low iron but containing EDTA (see Table 1). EDTA and citrate are to be used as chelators (see discussion). It is also disclosed that Y0 do not grow well in the protein free medium ABC whereas they do well in the mixture of media of the article.

D8 is an authors' publication where they replace transferrin with a range of the chelator tropolone and ferric ammonium citrate concentrations. No medium without tropolone was used. NSO myeloma cell lines were used for antibody production.

None of the documents cited in the International Search Report discloses the claimed subject-matter. The current set of claims is thus considered novel over these documents.

2. Inventive step (Art. 33(3) PCT)

D2 can be identified as the closest prior art since this document discloses the growth of myeloma cells in a protein-free medium, i.e. in which transferrin has been replaced with iron citrate. The subject-matter of independent claims 1, 10, 17, 26, 33 and 42 differs from this teaching in that the concentrations of iron used are much lower and that cells are grown under agitated conditions. Surprisingly and against the teaching of e.g. D6, myeloma cells can be grown in the absence of transferrin, a lipophilic or nitrogen-containing chelator with low concentration of iron and under agitated culture conditions. None of the documents cited in the International Search Report would have allowed the skilled person to achieve the subject-matter of the present application in an obvious manner. Therefore, inventive step can be acknowledged for the present set of claims.

3. Industrial applicability (Art. 33(4) PCT)

The present set of claims concerns industrially applicable subject-matter.